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TOXICITY STUDIES OF FORMALDEHYDE MONOMETHYLHYDRAZONE

W. C. KELLER, M. E. ANDERSEN,

R. H. BRUNER, J. P. MURPHY, AND K.C. BACK

TOXIC HAZARDS DIVISIONS
AIR FORCE AEROSPACE MEDICAL RESEARCH LABORATORY
WRIGHT-PATTERSON AFB, OH 45433

T. R. BOOSINGER

HQ AIR RESERVE PERSONNEL CENTER
DENVER, CO 80208

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AIR FORCE AEROSPACE MEDICAL RESEARCH LABORATORY
AEROSPACE MEDICAL DIVISION
AIR FORCE SYSTEMS COMMAND
WRIGHT-PATTERSON AIR FORCE BASE, OHIO 45433

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The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

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FOR THE COMMANDER

Roger C. Inman

ROGER C. INMAN, Colonel, USAF
Chief
Toxic Hazards Division
Air Force Aerospace Medical Research Laboratory

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* T. R. Boosinger (HQ Air Reserve Personnel Center, Denver CO 80208)

AFAMRL Primary Investigator: Major William C. Keller, AFAMRL/THT,
Tele: 513 255-3364

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20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

The toxicity of formaldehyde monomethylhydrazone (FMH) has been studied. The LD_{50} of FMH in the mouse was 42 mg/kg. Signs of acute toxicity were tremors and violent convulsions. Pyridoxine effectively prevented these convulsions. Reaction of FMH and DMBA appears similar to monomethylhydrazine (MMH) suggesting past errors in quantitating MMH using the spectrophotometric method. The high dose group (20 mg/kg) of mice in the short term toxicity study had an elevated serum aspartate aminotransferase (SGOT) but did not develop demonstratable histopathologic lesions involving the liver.

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PREFACE

This research was performed in the Toxicology Branch, Toxic Hazards Division, Aerospace Medical Research Laboratory from January 1979 through June 1982. It was performed in support of Project 6302, "Occupational and Environmental Toxic Hazards in Air Force Operations;" Task 630208, "Toxicology of Aerospace Fuels;" Work Unit 63020804, "Chronic Toxicology of Hydrazine Strategic Missile Fuels".

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INTRODUCTION

Monomethylhydrazine (MMH) and unsymmetrical dimethylhydrazine (UDMH) are used as missile propellants by the Air Force. Formaldehyde monomethylhydrazine (FMH) has been identified as a degradation product of MMH (Zirrolli et al., 1980) and as a product, along with formaldehyde dimethylhydrazine (FDH), of UDMH neutralization with hypochlorite (Mach and Baumgartner, 1978). Hypochlorite is preferred for neutralization of spills since the proposed alternative, hydrogen peroxide, produces considerable N-nitrosodimethylamine on reaction with UDMH.

Human exposure to FMH and FDH will most likely occur concurrently with exposure to MMH or UDMH, the hydrazones being either present as contaminants or as a consequence of a spill neutralization. The toxicity of both MMH and UDMH is well recognized but no data are available concerning the toxicity of FMH or FDH. The purpose of this research was to characterize the acute and short term toxicity of FMH. The toxicology of FDH will be reported in a later paper.

MATERIALS AND METHODS

FMH (Martin-Marietta, Denver CO) a dimerized crystalline material was stored under nitrogen. It turns brown upon continuous air exposure. Since only a few grams of the material were available, the numbers of animals used were necessarily small and studies were done in only one species, the mouse.

LD_{50} Determination: Male ICR mice (Charles River Breeding Laboratories) 18-31 gms were given intraperitoneal injections of MMH (Eastman, Rochester NY) or FMH dissolved in distilled water. Injection volume used was 0.01

ml/g body weight. Mice were individually observed for 8 hours following dose administration and then placed in group cages and held for two weeks. Group sizes were 15-18 mice/group. The LD_{50} was calculated using the log-dose probit analysis of Litchfield and Wilcoxon (1949). Preliminary range finding studies using smaller groups of mice were also done. These preliminary studies bracketed the LD_{50} for each chemical: MMH 24-36 mg/kg, and FMH 36-66 mg/kg. Four doses were used within each range.

Pyridoxine Treatment of FMH Toxicity: Male ICR mice were divided into 6 groups of 10 animals each. All groups were given 2 X LD_{50} of FMH. Each of the groups of mice was then given an ip pyridoxine (Hexa-Betalin, Eli Lilly Co.) dose of 0, 5, 10, 25, 50, or 100 mg/kg. The animals were placed in individual cages and observed for 8 hours. Time to convulsion was recorded. Survivors were group-caged at the end of 8 hours and killed at 48 hours.

Spectrophotometric Analysis of FMH: To determine the ability of the spectrophotometric method for serum MMH (Reynolds and Thomas, 1964) to also measure FMH, two equimolar aqueous solutions of FMH and MMH were prepared (0.05 mmol and 0.2 mmol). These aqueous samples were treated as serum in the method developed by Reynolds and Thomas except that the centrifugation step following addition of trichloroacetic acid was omitted. Absorbance at 485 nm was read at two minute intervals following addition of p-dimethylaminobenzaldehyde (DMBA) to each sample. Absorbance at equilibrium minus absorbance at each time interval versus time was plotted semilogarithmically.

Short Term FMH Toxicity: Three groups of 6 or 7 male ICR mice weighing 26-38 g were given intraperitoneal FMH doses of 0, 2, or 20 mg/kg for 14 consecutive days. The animals were examined and weighed daily. The animals were sacrificed by cervical dislocation 24 hours following the last injection. Tissues were examined grossly and microscopically for lesions. Blood samples were taken from the inferior vena cava at the time of sacrifice. Half the blood samples were examined for SGOT levels while a CBC was performed on samples from the remaining animals.

RESULTS

LD_{50} Determination: The LD_{50}'s for FMH and MMH were 42.0 (39-46) and 29 (27-30) mg/kg, respectively. Signs of toxicity were identical for FMH and MMH-treated mice. The animals exhibited depression, tremors, excitement, and violent convulsions in that sequence. Noise or other activity exacerbated the excitement and convulsions. The terminal convulsion usually progressed rapidly to general body rigidity and prosotonus (the head extended ventrally).

Pyridoxine Treatment of FMH Toxicity: Pyridoxine is an effective treatment for acute FMH toxicity in the mouse (Table 1). A dose related increase in time to convulsion was observed. In addition, a dose-related decrease in convulsions and mortality were also observed. No convulsions or mortality were observed at a pyridoxine dose of 100 mg/kg.

Table 1

Pyridoxine Treatment of FMH Toxicity[1]

Pyridoxine Dose (mg/kg)[2]	Time to Convulsion[3]	% Convulsing	% Mortality
0	68 ± 20	100	100
5	87 ± 18	90	60
10	141 ± 52	80	30
25	176 ± 49	30	10
50	144[4]	10	0
100	-	0	0

1 The FMH dose was 2X LD_{50}

2 Ten mice/group

3 Times listed as mean ± standard deviation. (minutes)

4 One mouse convulsed.

Spectrophotometric Analysis of FMH: Color development for equimolar solutions of FMH and MMH was nearly identical (Figure 1).

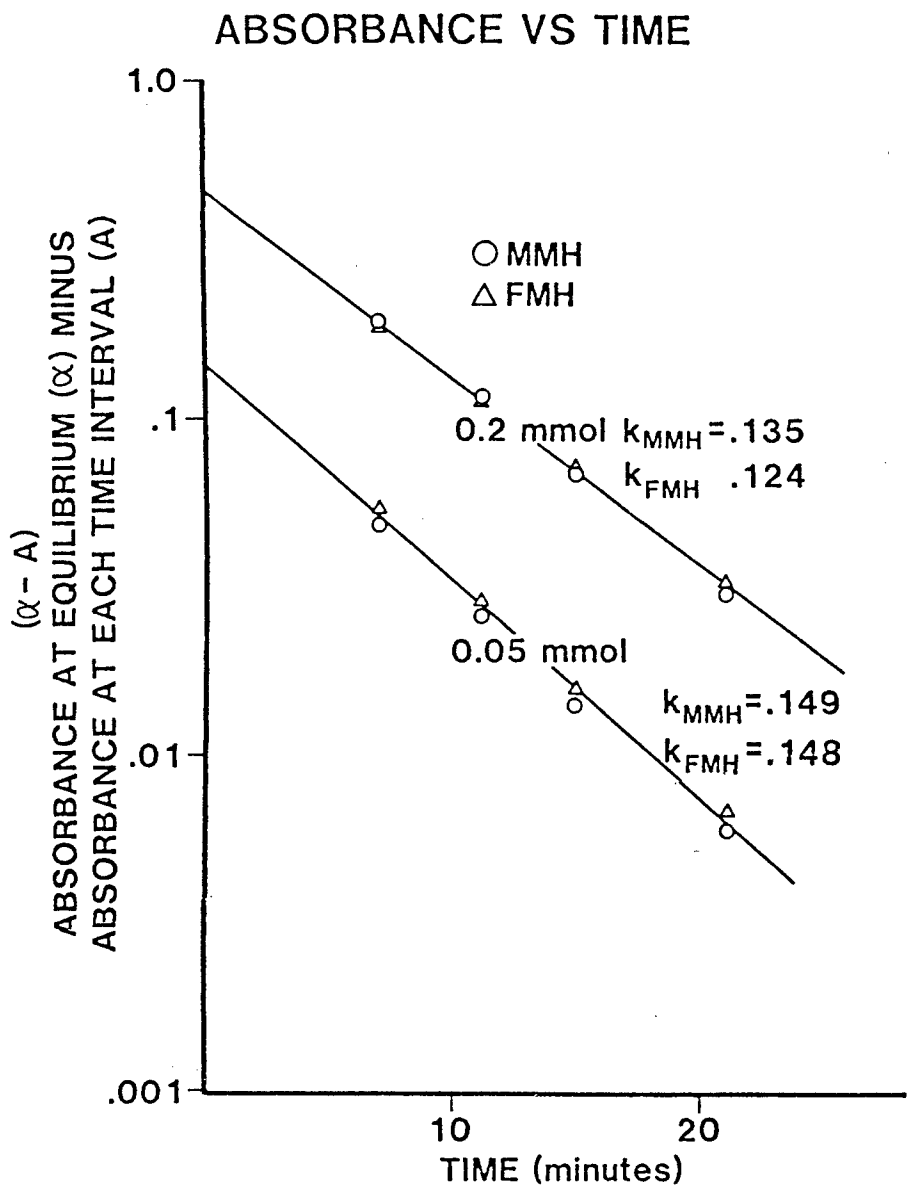

Figure 1. Comparison of the rate of reaction of DMBA and equimolar solutions of MMH and FMH by following color development.

Short Term FMH Toxicity: A dose-related decrease in hematocrit (HCT) and hemoglobin (HGB) and a dose-related increase in serum aspartate amino transferase (SGOT) occurred in mice treated with FMH (Table 2). A dose-related increase in hepatic lesions did not occur. No weight changes occurred in mice treated with FMH.

Table 2

Short Term FMH Toxicity[1]

	FMH Dose mg/kg		
	0	2	20
Hematocrit[2] %	44 ± 4	43 ± 0.6	33 ± 3
Hemoglobin[2] gm/DL	14.5 ± 0.4	14.7 ± 0.6	11.7 ± 0.7
Serum Aspartate Aminotransferase (SGOT)[2] IU	69 ± 12	80 ± 33	186 ± 116
Mean Weight Change (g)	+1	-1	+2

1 Daily ip injections for 14 days, animals sacrificed on day 15.

2 Values listed as mean ± standard deviation.

DISCUSSION

In contrast to the lack of data concerning FMH toxicity, numerous studies concerning the mechanism of action and treatment of acute MMH toxicity have been done. Many investigations have concentrated on the effect of MMH on some aspect of γ-aminobutyric acid (GABA) related metabolism and others on the use of pyridoxine to protect against MMH induced convulsions. The marked similarity of signs of MMH and FMH toxicity and a similar response to pyridoxine treatment suggests a similar mechanism of action, that is reaction

with pyridoxal 5-phosphate to form the pyridoxal-hydrazone, a potent GABA synthesis inhibitor or some related mechanism which ultimately decreases GABA levels. This also correlates with the similar chemical structures (Figure 2) of the two compounds. Since human FMH exposure will undoubtedly be concurrent with MMH exposure, the most recent information concerning therapeutics of MMH toxicity (George et al., 1982) should apply to FMH toxicity.

Figure 2. Chemical Structures of MMH and FMH.

The spectrophotometric finding of identical color development for FMH and MMH supports and expands previously reported work which suggested that FMH interfered with quantitation of MMH in an aqueous environment (Zirrolli et al., 1980). This suggests that previous spectrophotometric determinations of MMH in both environmental and toxicologic studies were reporting combined levels of MMH and FMH not MMH alone.

To test this hypothesis groups of 6 mice were given intraperitoneally a dose of 1.5 X LD_{50} of either MMH, FMH, or saline as a control. When the mice began to convulse they were sacrificed by cervical dislocation and a posterior vena caval blood sample taken. These samples were pooled to give a group sample and prepared and analyzed for MMH by the method of Reynolds and Thomas (1964). The absorbances for MMH, FMH, and control were 1.178, 0.426, and 0.029, respectively, thus supporting the proposition that FMH may be

spectrophotometrically quantitated as MMH. It should be noted that FMH lacks the free NH_2 group present in MMH (Figure 2), thus suggesting that other groups are capable of reacting with DMBA to yield color, or that a rapid exchange reaction may occur between the hydrazone and DMBA.

Histopathologic lesions were not found in mice exposed to FMH. This is in contrast to hepatic lesions reported in MMH exposed mice including centrilobular cholestasis, bile duct proliferation, and centrilobular hemosiderosis (Kroe, 1971). In addition, splenic and renal hemosiderosis were also observed in MMH exposed mice. Hepatic lesions have also been observed in formaldehyde dimethylhydrazone exposed mice (Keller et al., 1983). A possible reason for the lack of histopathologic lesions in the FMH treated mice is the relatively short exposure time (2 weeks) versus the MMH exposed mice (6 months). The elevated SGOT reported in FMH-treated mice also suggests possible hepatotoxicity which is not yet detectable histopathologically. The moderately depressed HCT and HGB are of questionable significance since the number of animals involved is very small and no hemosiderosis was reported. Hemolytic anemia as a consequence of MMH exposure (NIOSH, 1978) has been reported in some species, however, and any future subchronic or chronic toxicity studies with FMH should examine this potential toxic effect. In addition, the elevated SGOT observed in the high dose group of this study suggests that this compound may be hepatotoxic. Future chronic studies should be designed to examine this possibility.

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